(FILE 'HOME' ENTERED AT 11:18:16 ON 16 APR 2003)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 11:18:50 ON 16 APR 2003

E RUVKUN G?/AU

L1 76 S E4
L2 69 DUP REM L1 (7 DUPLICATES REMOVED)
L3 11 S L2 AND DAF-16
L4 11 SORT L3 PY
L5 254 S DAF-16
L6 117 DUP REM L5 (137 DUPLICATES REMOVED)
L7 9 S L6 AND PY<=1997
L8 9 SORT L7 PY
L9 80 S L6 AND (EXPRES? OR MODULA? OR INCREAS? OR DECREAS?)
L10 80 FOCUS L9 1-

=> d an ti so au ab l10 2 3 5 8

L10 ANSWER 2 OF 80 MEDLINE
AN 2001699838 MEDLINE
TI Regulation of C. elegans **DAF-16** and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway.
SO CURRENT BIOLOGY, (2001 Dec 11) 11 (24) 1950-7.
Journal code: 9107782. ISSN: 0960-9822.
AU Lee R Y; Hench J; Ruvkun G
AB C. elegans insulin-like signaling regulates metabolism, development, and life span. This signaling pathway negatively regulates the activity of the forkhead transcription factor **DAF-16**. **daf-16** encodes multiple isoforms that are **expressed** in distinct tissue types and are probable orthologs of human FKHRL1, FKHR, and AFX. We show that human FKHRL1 can partially replace **DAF-16**, proving the orthology. In mammalian cells, insulin and insulin-like growth factor signaling activate AKT/PKB kinase to negatively regulate the nuclear localization of **DAF-16** homologs (reviewed in). We show that the absence of AKT consensus sites on **DAF-16** is sufficient to cause dauer arrest in daf-2(+) animals, proving that **daf-16** is the major output of insulin signaling in C. elegans. FKHR, FKRHL1, and AFX may similarly be the major outputs of mammalian insulin signaling. daf-2 insulin signaling, via AKT kinases, negatively regulates **DAF-16** by controlling its nuclear localization. Surprisingly, we find that daf-7 TGF-beta signaling also regulates **DAF-16** nuclear localization specifically at the time when the animal makes the commitment between diapause and reproductive development. **daf-16** function is supported by the combined action of two distinct promoter/enhancer elements, whereas the coding sequences of two major **DAF-16** isoforms are interchangeable. Together, these observations suggest that the combined effects of transcriptional and posttranslational regulation of **daf-16** transduce insulin-like signals in C. elegans and perhaps more generally.

L10 ANSWER 3 OF 80 MEDLINE
AN 1998013175 MEDLINE
TI The Fork head transcription factor **DAF-16** transduces insulin-like metabolic and longevity signals in C. elegans.
SO NATURE, (1997 Oct 30) 389 (6654) 994-9.
Journal code: 0410462. ISSN: 0028-0836.
AU Ogg S; Paradis S; Gottlieb S; Patterson G I; Lee L; Tissenbaum H A; Ruvkun G
AB In mammals, insulin signalling regulates glucose transport together with the **expression** and activity of various metabolic enzymes. In the nematode Caenorhabditis elegans, a related pathway regulates metabolism, development and longevity. Wild-type animals enter the developmentally arrested dauer stage in response to high levels of a secreted pheromone, accumulating large amounts of fat in their intestines and hypodermis. Mutants in DAF-2 (a homologue of the mammalian insulin receptor) and AGE-1 (a homologue of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) arrest development at the dauer stage. Moreover, animals bearing weak or temperature-sensitive mutations in daf-2

and age-1 can develop reproductively, but nevertheless show **increased** energy storage and longevity. Here we show that null mutations in **daf-16** suppress the effects of mutations in daf-2 or age-1; lack of **daf-16** bypasses the need for this insulin receptor-like signalling pathway. The principal role of DAF-2/AGE-1 signalling is thus to antagonize **DAF-16**. **daf-16** is widely **expressed** and encodes three members of the Fork head family of transcription factors. The DAF-2 pathway acts synergistically with the pathway activated by a nematode TGF-beta-type signal, DAF-7, suggesting that **DAF-16** cooperates with nematode SMAD proteins in regulating the transcription of key metabolic and developmental control genes. The probable human orthologues of **DAF-16**, FKHR and AFX, may also act downstream of insulin signalling and cooperate with TGF-beta effectors in mediating metabolic regulation. These genes may be dysregulated in diabetes.

L10 ANSWER 5 OF 80 MEDLINE
AN 2001698389 MEDLINE
TI **DAF-16**-dependent and independent **expression** targets of DAF-2 insulin receptor-like pathway in Caenorhabditis elegans include FKBPs.
SO JOURNAL OF MOLECULAR BIOLOGY, (2001 Dec 14) 314 (5) 1017-28. Journal code: 2985088R. ISSN: 0022-2836.
AU Yu H; Larsen P L
AB The daf-2 insulin-like receptor pathway regulates development and life-span in Caenorhabditis elegans. Reduced DAF-2 signaling leads to changes in downstream targets via the **daf-16** gene, a fork-head transcription factor which is regulated by DAF-2, and results in extended life-span. Here, we describe the first identification of genes whose **expression** is controlled by the DAF-2 signaling cascade. dao-1, dao-2, dao-3, dao-4, dao-8 and dao-9 are down-regulated in daf-2 mutant adults compared to wild-type adults, whereas dao-5, dao-6 and dao-7 are up-regulated. The latter genes are negatively regulated by DAF-2 signaling and positively regulated by **DAF-16**. Positive regulation by DAF-2 on dao-1, dao-4 and dao-8 was mediated by **DAF-16**, whereas **daf-16** mediates only part of DAF-2 signaling for dao-2 and dao-9. Regulation by DAF-2 is most likely **DAF-16** independent for dao-3 and hsp-90. RNA levels of dao-5 and dao-6 showed elevated **expression** in daf-2 adults, as well as being strongly **expressed** in dauer larvae. In contrast, hsp-90 transcript levels are low in daf-2 mutant adults though they are enriched in dauer larvae, indicating overlapping but not identical mechanisms of efficient life maintenance in stress-resistant dauer larvae and long-lived daf-2 mutant adults. dao-1, dao-8 and dao-9 are homologs of the FK506 binding proteins that interact with the mammalian insulin pathway. dao-3 encodes a putative methylenetetrahydrofolate dehydrogenase. DAO-5 shows 33 % identity with human nucleolar phosphoprotein P130. dao-7 is similar to the mammalian ZFP36 protein. Distinct regulatory patterns of dao genes implicate their diverse positions within the signaling network of DAF-2 pathway, and suggest they have unique contributions to development, metabolism and longevity.

L10 ANSWER 8 OF 80 MEDLINE
AN 1998028757 MEDLINE
TI **daf-16**: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans.
SO SCIENCE, (1997 Nov 14) 278 (5341) 1319-22. Journal code: 0404511. ISSN: 0036-8075.
AU Lin K; Dorman J B; Rodan A; Kenyon C
AB The wild-type Caenorhabditis elegans nematode ages rapidly, undergoing development, senescence, and death in less than 3 weeks. In contrast, mutants with reduced activity of the gene daf-2, a homolog of the insulin and insulin-like growth factor receptors, age more slowly than normal and live more than twice as long. These mutants are active and fully fertile and have normal metabolic rates. The life-span extension caused by daf-2 mutations requires the activity of the gene **daf-16**. **daf-16** appears to play a unique role in life-span

regulation and encodes a member of the hepatocyte nuclear factor 3 (HNF-3)/forkhead family of transcriptional regulators. In humans, insulin down-regulates the **expression** of certain genes by antagonizing the activity of HNF-3, raising the possibility that aspects of this regulatory system have been conserved.

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L7 9 S L6 AND PY<=1997
L8 9 SORT L7 PY

=> d an ti so au ab pi l8 1-9

L8 ANSWER 1 OF 9 MEDLINE
AN 92120509 MEDLINE
TI Genetic analysis of chemosensory control of dauer formation in Caenorhabditis elegans.
SO GENETICS, **(1992 Jan)** 130 (1) 105-23.
Journal code: 0374636. ISSN: 0016-6731.
AU Vowels J J; Thomas J H
AB Dauer larva formation in Caenorhabditis elegans is controlled by chemosensory cells that respond to environmental cues. Genetic interactions among mutations in 23 genes that affect dauer larva formation were investigated. Mutations in seven genes that cause constitutive dauer formation, and mutations in 16 genes that either block dauer formation or result in the formation of abnormal dauers, were analyzed. Double mutants between dauer-constitutive and dauer-defective mutations were constructed and characterized for their capacity to form dauer larvae. Many of the genes could be interpreted to lie in a simple linear epistasis pathway. Three genes, **daf-16**, daf-18 and daf-20, may affect downstream steps in a branched part of the pathway. Three other genes, daf-2, daf-3 and daf-5, displayed partial or complex epistasis interactions that were difficult to interpret as part of a simple linear pathway. Dauer-defective mutations in nine genes cause structurally defective chemosensory cilia, thereby blocking chemosensation. Mutations in all nine of these genes appear to fall at a single step in the epistasis pathway. Dauer-constitutive mutations in one gene, daf-11, were strongly suppressed for dauer formation by mutations in the nine cilium-structure genes. Mutations in the other six dauer-constitutive genes caused dauer formation despite the absence of functional chemosensory endings. These results suggest that daf-11 is directly involved in chemosensory transduction essential for dauer formation, while the other Daf-c genes play roles downstream of the chemosensory step.

L8 ANSWER 2 OF 9 MEDLINE
AN 94067343 MEDLINE
TI A C. elegans mutant that lives twice as long as wild type.
SO NATURE, **(1993 Dec 2)** 366 (6454) 461-4.
Journal code: 0410462. ISSN: 0028-0836.
AU Kenyon C; Chang J; Gensch E; Rudner A; Tabtiang R
AB We have found that mutations in the gene daf-2 can cause fertile, active, adult Caenorhabditis elegans hermaphrodites to live more than twice as long as wild type. This lifespan extension, the largest yet reported in any organism, requires the activity of a second gene, **daf-16**. Both genes also regulate formation of the dauer larva, a developmentally arrested larval form that is induced by crowding and starvation and is very long-lived. Our findings raise the possibility that the longevity of the dauer is not simply a consequence of its arrested growth, but instead results from a regulated lifespan extension mechanism that can be uncoupled from other aspects of dauer formation. daf-2 and **daf-16** provide entry points into understanding how lifespan can be extended.

L8 ANSWER 3 OF 9 MEDLINE
AN 94333774 MEDLINE
TI daf-2, **daf-16** and daf-23: genetically interacting genes controlling Dauer formation in Caenorhabditis elegans.

SO GENETICS, **(1994 May)** 137 (1) 107-20.
Journal code: 0374636. ISSN: 0016-6731.

AU Gottlieb S; Ruvkun G

AB Under conditions of high population density and low food, Caenorhabditis elegans forms an alternative third larval stage, called the dauer stage, which is resistant to desiccation and harsh environments. Genetic analysis of some dauer constitutive (Daf-c) and dauer defective (Daf-d) mutants has revealed a complex pathway that is likely to function in particular neurons and/or responding tissues. Here we analyze the genetic interactions between three genes which comprise a branch of the dauer formation pathway that acts in parallel to or downstream of the other branches of the pathway, the Daf-c genes daf-2 and daf-23 and the Daf-d gene **daf-16**. Unlike mutations in other Daf-c genes, mutations in both daf-2 and daf-23 cause non-conditional arrest at the dauer stage. Our epistasis analysis suggests that daf-2 and daf-23 are functioning at a similar point in the dauer pathway. First, mutations in daf-2 and daf-23 are epistatic to mutations in the same set of Daf-d genes. Second, daf-2 and daf-23 mutants are suppressed by mutations in **daf-16**. Mutations in **daf-16** do not suppress any of the other Daf-c mutants as efficiently as they suppress daf-2 and daf-23 mutants. Third, double mutants between either daf-2 or daf-23 and several other daf-d mutants exhibit an unusual interaction. Based on these results, we present a model for the function of daf-2, daf-23 and **daf-16** in dauer formation.

L8 ANSWER 4 OF 9 MEDLINE

AN 96170778 MEDLINE

TI The age-1 and daf-2 genes function in a common pathway to control the lifespan of Caenorhabditis elegans.

SO GENETICS, **(1995 Dec)** 141 (4) 1399-406.
Journal code: 0374636. ISSN: 0016-6731.

AU Dorman J B; Albinder B; Shroyer T; Kenyon C

AB Recessive mutations in two genes, daf-2 and age-1, extend the lifespan of Caenorhabditis elegans significantly. The daf-2 gene also regulates formation of an alternative developmental state called the dauer. Here we asked whether these two genes function in the same or different lifespan pathways. We found that the longevity of both age-1 and daf-2 mutants requires the activities of the same two genes, **daf-16** and daf-18. In addition, the daf-2(e1370); age-1(hx546) double mutant did not live significantly longer than the daf-2 single mutant. We also found that, like daf-2 mutations, the age-1(hx546) mutation affects certain aspects of dauer formation. These findings suggest that age-1 and daf-2 mutations do act in the same lifespan pathway and extend lifespan by triggering similar if not identical processes.

L8 ANSWER 5 OF 9 MEDLINE

AN 95309673 MEDLINE

TI Genes that regulate both development and longevity in Caenorhabditis elegans.

SO GENETICS, **(1995 Apr)** 139 (4) 1567-83.
Journal code: 0374636. ISSN: 0016-6731.

AU Larsen P L; Albert P S; Riddle D L

AB The nematode Caenorhabditis elegans responds to conditions of overcrowding and limited food by arresting development as a dauer larva. Genetic analysis of mutations that alter dauer larva formation (daf mutations) is presented along with an updated genetic pathway for dauer vs. nondauer development. Mutations in the daf-2 and daf-23 genes double adult life span, whereas mutations in four other dauer-constitutive genes positioned in a separate branch of this pathway (daf-1, daf-4, daf-7 and daf-8) do not. The increased life spans are suppressed completely by a **daf-16** mutation and partially in a daf-2; daf-18 double mutant. A genetic pathway for determination of adult life span is presented based on the same strains and growth conditions used to characterize Daf phenotypes. Both dauer larva formation and adult life span are affected in daf-2; daf-12 double mutants in an allele-specific manner. Mutations in daf-12 do not extend adult life span, but certain combinations of daf-2 and daf-12 mutant alleles nearly quadruple it. This synergistic effect, which does not equivalently extend the fertile period, is the largest genetic extension of life span yet observed in a metazoan.

L8 ANSWER 6 OF 9 MEDLINE
AN 96400918 MEDLINE
TI A genetic pathway conferring life extension and resistance to UV stress in Caenorhabditis elegans.
SO GENETICS, **(1996 Jul)** 143 (3) 1207-18.
Journal code: 0374636. ISSN: 0016-6731.
AU Murakami S; Johnson T E
AB A variety of mechanisms have been proposed to explain the extension of adult life span (Age) seen in several mutants in Caenorhabditis elegans (age-1: an altered aging rate; daf-2 and daf-23: activation of a dauer-specific longevity program; spe-26: reduced fertility; clk-1: an altered biological clock). Using an assay for ultraviolet (UV) resistance in young adult hermaphrodites (survival after UV irradiation), we observed that all these Age mutants show increased resistance to UV. Moreover, mutations in **daf-16** suppressed the UV resistance as well as the increased longevity of all the Age mutants. In contrast to the multiple mechanisms initially proposed, these results suggest that a single, **daf-16**-dependent pathway, specifies both extended life span and increased UV resistance. The mutations in **daf-16** did not alter the reduced fertility of spe-26 and interestingly a **daf-16** mutant is more fertile than wild type. We propose that life span and some aspects of stress resistance are jointly negatively regulated by a set of gerontogenes (genes whose alteration causes life extension) in C. elegans.

L8 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2003 ACS
AN 1996:303001 CAPLUS
DN 125:5796
TI Determination of life-span in Caenorhabditis elegans by four Clock genes
SO Science (Washington, D. C.) (**1996**), 272(5264), 1010-1013
CODEN: SCIEAS; ISSN: 0036-8075
AU Lakowski, Bernard; Hekimi, Siegfried
AB The nematode worm Caenorhabditis elegans is a model system for the study of the genetic basis of aging. Maternal-effect mutations in four genes-clk-1, clk-2, clk-3, and gro-1-interact genetically to det. both the duration of development and life-span. Anal. of the phenotypes of these mutants suggests the existence of a general physiol. clock in the worm. Mutations in certain genes involved in dauer formation (an alternative larval stage induced by adverse conditions in which development is arrested) can also extend life-span, but the life extension of Clock mutants appears to be independent of these genes. The daf-2(e1370) clk-1(e2519) worms, which carry life-span-extending mutations from two different pathways, live nearly five times as long as wild-type worms.

L8 ANSWER 8 OF 9 MEDLINE
AN 1998028757 MEDLINE
TI **daf-16**: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans.
SO SCIENCE, **(1997 Nov 14)** 278 (5341) 1319-22.
Journal code: 0404511. ISSN: 0036-8075.
AU Lin K; Dorman J B; Rodan A; Kenyon C
AB The wild-type Caenorhabditis elegans nematode ages rapidly, undergoing development, senescence, and death in less than 3 weeks. In contrast, mutants with reduced activity of the gene daf-2, a homolog of the insulin and insulin-like growth factor receptors, age more slowly than normal and live more than twice as long. These mutants are active and fully fertile and have normal metabolic rates. The life-span extension caused by daf-2 mutations requires the activity of the gene **daf-16**. **daf-16** appears to play a unique role in life-span regulation and encodes a member of the hepatocyte nuclear factor 3 (HNF-3)/forkhead family of transcriptional regulators. In humans, insulin down-regulates the expression of certain genes by antagonizing the activity of HNF-3, raising the possibility that aspects of this regulatory system have been conserved.

L8 ANSWER 9 OF 9 MEDLINE
AN 1998013175 MEDLINE
TI The Fork head transcription factor **DAF-16** transduces insulin-like metabolic and longevity signals in C. elegans.
SO NATURE, **(1997 Oct 30)** 389 (6654) 994-9.

Journal code: 0410462. ISSN: 0028-0836.

AU Ogg S; Paradis S; Gottlieb S; Patterson G I; Lee L; Tissenbaum H A; Ruvkun G

AB In mammals, insulin signalling regulates glucose transport together with the expression and activity of various metabolic enzymes. In the nematode Caenorhabditis elegans, a related pathway regulates metabolism, development and longevity. Wild-type animals enter the developmentally arrested dauer stage in response to high levels of a secreted pheromone, accumulating large amounts of fat in their intestines and hypodermis. Mutants in DAF-2 (a homologue of the mammalian insulin receptor) and AGE-1 (a homologue of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) arrest development at the dauer stage. Moreover, animals bearing weak or temperature-sensitive mutations in daf-2 and age-1 can develop reproductively, but nevertheless show increased energy storage and longevity. Here we show that null mutations in **daf-16** suppress the effects of mutations in daf-2 or age-1; lack of **daf-16** bypasses the need for this insulin receptor-like signalling pathway. The principal role of DAF-2/AGE-1 signalling is thus to antagonize **DAF-16**. **daf-16** is widely expressed and encodes three members of the Fork head family of transcription factors. The DAF-2 pathway acts synergistically with the pathway activated by a nematode TGF-beta-type signal, DAF-7, suggesting that **DAF-16** cooperates with nematode SMAD proteins in regulating the transcription of key metabolic and developmental control genes. The probable human orthologues of **DAF-16**, FKHR and AFX, may also act downstream of insulin signalling and cooperate with TGF-beta effectors in mediating metabolic regulation. These genes may be dysregulated in diabetes.

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L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1997:702845 CAPLUS
DN 128:20747
TI The Fork head transcription factor **DAF-16** transduces insulin-like metabolic and longevity signals in C. elegans
SO Nature (London) (1997), 389(6654), 994-999
CODEN: NATUAS; ISSN: 0028-0836
AU Ogg, Scott; Paradis, Suzanne; Gottlieb, Shoshanna; Patterson, Garth I.; Lee, Linda; Tissenbaum, Heidi A.; **Ruvkun, Gary**
AB In mammals, insulin signaling regulates glucose transport together with the expression and activity of various metabolic enzymes. In the nematode Caenorhabditis elegans, a related pathway regulates metab., development and longevity. Wild-type animals enter the developmentally arrested dauer stage in response to high levels of a secreted pheromone, accumulating large amts. of fat in their intestines and hypodermis. Mutants in DAF-2 (a homolog of the mammalian insulin receptor) and AGE-1 (a homolog of the catalytic subunit of mammalian phosphatidylinositol 3-OH kinase) arrest development at the dauer stage. Moreover, animals bearing weak or temp.-sensitive mutations in daf-2 and age-1 can develop reproductively, but nevertheless show increased energy storage and longevity. Null mutations in **daf-16** suppress the effects of mutations in daf-2 or age-1; lack of **daf-16** bypasses the need for this insulin receptor-like signaling pathway. **DAF-16** is widely expressed and encodes three members of the Fork head family of transcription factors. The DAF-2 pathway acts synergistically with the pathway activated by a nematode TGF-.beta.-type signal, DAF-7, suggesting that **DAF-16** cooperates with nematode SMAD proteins in regulating the transcription of key metabolic and developmental control genes. The probable human orthologs of **DAF-16**, FKHR and AFX, may also act downstream of insulin signaling and cooperate with TGF-.beta. effectors in mediating metabolic regulation. These genes may be dysregulated in diabetes.

L4 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1999:37224 CAPLUS
DN 130:220650
TI The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway
SO Molecular Cell (1998), 2(6), 887-893
CODEN: MOCEFL; ISSN: 1097-2765
AU Ogg, Scott; **Ruvkun, Gary**
AB An insulin-like signaling pathway, from the DAF-2 receptor, the AGE-1 phosphoinositide 3-kinase, and the AKT-1/AKT-2 serine/threonine kinases to the **DAF-16** Fork head transcription factor, regulates the metab., development, and life span of Caenorhabditis elegans. Inhibition of daf-18 gene activity bypasses the normal requirement for AGE-1 and partially bypasses the need for DAF-2 signaling. The suppression of age-1 mutations by a daf-18 mutation depends on AKT-1/AKT-2 signaling, showing that DAF-18 acts between AGE-1 and the AKT input to **DAF-16** transcriptional regulation. Daf-18 encodes a homolog of the human tumor suppressor PTEN (MMAC1/TEP1), which has 3-phosphatase activity toward phosphatidylinositol 3,4,5-trisphosphate (PIP3). DAF-18 PTEN may normally limit AKT-1 and AKT-2 activation by decreasing PIP3 levels. The action of daf-18 in this metabolic control pathway suggests that mammalian PTEN may modulate insulin signaling and may be variant in diabetic pedigrees.

L4 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1998:761816 CAPLUS
DN 130:29188
TI Therapeutic and diagnostic tools for impaired glucose tolerance conditions based on the dauer polypeptides and genes of Caenorhabditis elegans
SO PCT Int. Appl., 202 pp.
CODEN: PIXXD2
IN **Ruvkun, Gary**; Kimura, Koutarou; Patterson, Garth; Ogg, Scott; Paradis, Suzanne; Tissenbaum, Heidi; Morris, Jason; Koweek, Allison; Pierce, Sarah
AB Disclosed herein are novel genes and methods for the screening of therapeutics useful for treating impaired glucose tolerance conditions, as

well as diagnostics and therapeutic compns. for identifying or treating such conditions. The Caenorhabditis elegans metabolic regulatory genes daf-2 and age-1 encode homologs of the mammalian insulin receptor/phosphoinositide 3-kinase signaling pathway proteins, resp. In addn., the **DAF-16** forkhead protein represents the major transcriptional output of this insulin signaling pathway. Dysregulation of the **DAF-16** transcription factor in the absence of insulin signaling leads to metabolic defects; inactivation of **DAF-16** reverses the metabolic defects caused by lack of insulin signaling in C. elegans. Finally, the C. elegans daf-7, da-1, daf-4, daf-8, daf-14, and daf-3 genes encode neuroendocrine/target tissue transforming growth factor-.beta. type signal transduction mols. that genetically interact with the insulin signaling pathway. Metabolic defects cause by lack of neuroendocrine TGF-.beta. signals can be reversed by inactivation of the DAF-3 transcription factor. The C. elegans daf genes are excellent candidate genes and proteins for human disease assocd. with glucose intolerance, e.g., diabetes, obesity, and atherosclerosis. The human homologs of these daf genes and proteins mediate insulin signaling in normal people and may be defective or mis-regulated in diabetics. Moreover, there are at least 2 classes of type II diabetics: those with defects in the TGF-.beta. signaling genes, and those with defects in insulin signaling genes. Exemplary sequences and functional characteristics are provided for the C. elegans daf homologs of the human genes: daf-2, daf-3 (3 differentially spliced isoforms), **daf-16** (2 differentially spliced isoforms), age-1, and pdk-1 (two spliced isoforms).

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9851351	A1	19981119	WO 1998-US10080	19980515
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
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	US 6225120	B1	20010501	US 1997-857076	19970515
	AU 9874941	A1	19981208	AU 1998-74941	19980515
	AU 752962	B2	20021003		
	EP 1019092	A1	20000719	EP 1998-922382	19980515
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	JP 2002511747	T2	20020416	JP 1998-549639	19980515
	US 2001029617	A1	20011011	US 1998-205658	19981203
	US 2002037585	A1	20020328	US 2001-844353	20010427

L4 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1998:577164 CAPLUS
DN 129:287966
TI Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the **DAF-16** transcription factor
SO Genes & Development (1998), 12(16), 2488-2498
CODEN: GEDEEP; ISSN: 0890-9369
AU Paradis, Suzanne; **Ruvkun, Gary**
AB A neurosecretory pathway regulates a reversible developmental arrest and metabolic shift at the Caenorhabditis elegans dauer larval stage. Defects in an insulin-like signaling pathway cause arrest at the dauer stage. We show here that two C. elegans Akt/PkB homologs, akt-1 and akt-2, transduce insulin receptor-like signals that inhibit dauer arrest and that AKT-1 and AKT-2 signaling are indispensable for insulin receptor-like signaling in C. elegans. A loss-of-function mutation in the Fork head transcription factor **DAF-16** relieves the requirement for Akt/PKB signaling, which indictes that AKT-1 and AKT-2 function primarily to antagonize **DAF-16**. This is the first evidence that the major target of Akt/PKB signaling is a transcription factor. An activation mutation in akt-1, revealed by a genetic screen, as well as increased dosage of wild-type akt-1 relieves the requirements for signaling from AGE-1 PI3K, which acts downstream of the DAF-02

insulin/IGF-1 receptor homol. This demonstrates that Akt/PKB activity is not necessarily dependent on AGE-1 PI3K activity. Akt-1 and akt-2 are expressed in overlapping patterns in the nervous system and in tissues that are remodeled during dauer formation.

L4 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1998:463129 CAPLUS
DN 129:228428
TI An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans
SO Genetics (1998), 148(2), 703-717
CODEN: GENTAE; ISSN: 0016-6731
AU Tissenbaum, Heidi A.; **Ruvkun, Gary**
AB Mutations in daf-2 and age-1 cause a dramatic increase in longevity as well as developmental arrest at the dauer diapause stage in Caenorhabditis elegans. Daf-2 and age-1 encode components of an insulin-like signaling pathway. Both daf-2 and age-1 act at a similar point in the genetic epistasis pathway for dauer arrest and longevity and regulate the activity of the **daf-16** gene. Mutations in **daf-16** cause a dauer-defective phenotype and are epistatic to the diapause arrest and life span extension phenotypes of daf-2 and age-1 mutants. Mutations in this pathway also affect fertility and embryonic development. Weak daf-2 alleles, and maternally rescued age-1 alleles that cause life span extension but do not arrest at the dauer stage, also reduce fertility and viability. The authors find that age-1(hx546) has reduced both maternal and zygotic age-1 activity. **Daf-16** mutations suppress all of the daf-2 and age-1 phenotypes, including dauer arrest, life span extension, reduced fertility, and viability defects. These data show that insulin signaling, mediated by DAF-2 through the AGE-1 phosphatidylinositol-3-hydroxykinase, regulates reprodn. and embryonic development, as well as dauer diapause and life span, and that **DAF-16** transduces these signals. The regulation of fertility, life span, and metab. by an insulin-like signaling pathway is similar to the endocrine regulation of metab. and fertility by mammalian insulin signaling.

L4 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1999:382232 CAPLUS
DN 131:182560
TI A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans
SO Genes & Development (1999), 13(11), 1438-1452
CODEN: GEDEEP; ISSN: 0890-9369
AU Paradis, Suzanne; Ailion, Michael; Toker, Alex; Thomas, James H.; **Ruvkun, Gary**
AB An insulin receptor-like signaling pathway regulates Caenorhabditis elegans metab., development, and longevity. Inactivation of the insulin receptor homolog DAF-2, the AGE-1 PI3K, or the AKT-1 and AKT-2 kinases causes a developmental arrest at the dauer stage. A null mutation in the **daf-16** Fork head transcription factor alleviates the requirement for signaling through this pathway. We show here that a loss-of-function mutation in pdk-1, the C. elegans homolog of the mammalian Akt/PKB kinase PDK1, results in constitutive arrest at the dauer stage and increased life span; these phenotypes are suppressed by a loss of function mutation in **daf-16**. An activating mutation in pdk-1 or overexpression of wild-type pdk-1 relieves the requirement for AGE-1 P13K signaling. Therefore, pdk-1 activity is both necessary and sufficient to propagate AGE-1 PI3K signals in the DAF-2 insulin receptor-like signaling pathway. The activating mutation in pdk-1 requires akt-1 and akt-2 gene activity in order to suppress the dauer arrest phenotype of age-1. This indicates that the major function of C. elegans PDKI is to transduce signals from AGE-1 to AKT-1 and AKT-2. The activating pdk-1 mutation is located in a conserved region of the kinase domain; the equiv. amino acid substitution in human PDK1 activates its kinase activity toward mammalian Akt/PKB.

L4 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 2000:664827 CAPLUS
DN 134:158393
TI **DAF-16** recruits the CREB-binding protein coactivator

complex to the insulin-like growth factor binding protein 1 promoter in HepG2 cells

SO Proceedings of the National Academy of Sciences of the United States of America (2000), 97(19), 10412-10417
CODEN: PNASA6; ISSN: 0027-8424

AU Nasrin, Nargis; Ogg, Scott; Cahill, Catherine M.; Biggs, William; Nui, Simin; Dore, Justin; Calvo, Dominica; Shi, Yang; **Ruvkun, Gary**; Alexander-Bridges, Maria C.

AB Insulin neg. regulates expression of the insulin-like growth factor binding protein 1 (IGFBP-1) gene by means of an insulin-responsive element (IRE) that also contributes to glucocorticoid stimulation of this gene. We find that the Caenorhabditis elegans protein **DAF-16** binds the IGFBP-1.cntdot.IRE with specificity similar to that of the forkhead (FKH) factor(s) that act both to enhance glucocorticoid responsiveness and to mediate the neg. effect of insulin at this site. In HepG2 cells, **DAF-16** and its mammalian homologs, FKHR, FKHRL1, and AFX, activate transcription through the IGFBP-1.cntdot.IRE; this effect is inhibited by the viral oncoprotein E1A, but not by mutants of E1A that fail to interact with the coactivator p300/CREB-binding protein (CBP). We show that **DAF-16** and FKHR can interact with both the KIX and E1A/5RC interaction domains of p300/CBP, as well as the steroid receptor coactivator (SRC). A C-terminal deletion mutant of **DAF-16** that is nonfunctional in C. elegans fails to bind the KIX domain of CBP, fails to activate transcription through the IGFBP-1.cntdot.IRE, and inhibits activation of the IGFBP-1 promoter by glucocorticoids. Thus, the interaction of **DAF-16** homologs with the KIX domain of CBP is essential to basal and glucocorticoid-stimulated transactivation. Although AFX interacts with the KIX domain of CBP, it does not interact with SRC and does not respond to glucocorticoids or insulin. Thus, we conclude that **DAF-16** and FKHR act as accessory factors to the glucocorticoid response, by recruiting the p300/CBP/SRC coactivator complex to an FKH factor site in the IGFBP-1 promoter, which allows the cell to integrate the effects of glucocorticoids and insulin on genes that carry this site.

L4 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2003 ACS

AN 2000:384548 CAPLUS

DN 133:39116

TI Genes and polypeptides involved in insulin signaling pathways for glucose tolerance, obesity, and longevity and their uses as therapeutic and diagnostic tools

SO PCT Int. Appl., 402 pp.
CODEN: PIXXD2

IN **Ruvkun, Gary**; Ogg, Scott

AB Disclosed herein are novel genes and methods for the screening of therapeutics useful for treating impaired glucose tolerance conditions, as well as diagnostics and therapeutic compns. for identifying or treating such conditions. The Caenorhabditis elegans metabolic regulatory genes daf-2 and age-1 encode homologs of the mammalian insulin receptor/phosphoinositol 3-kinase signaling pathway proteins, resp. Also, the C. elegans PKB kinase and AKT kinase act downstream of these genes, as their mammalian homologs act downstream of insulin signaling. The C. elegans PTEN lipid phosphatase homolog, DAF-18, acts upstream of AKT in this signaling pathway. Further, the **DAF-16** forkhead protein represents the major transcriptional output of this insulin signaling pathway. Addnl. evidence indicates that the **DAF-16**, DAF-3, DAF-8, and DAF-14 transcriptional outputs of converging signaling pathways regulate metab. The congruence between the C. elegans and mammalian insulin signaling pathways strongly supports the contention that new genes identified in the C. elegans pathway also act in mammalian insulin signaling. Exemplary sequences and functional characteristics of the C. elegans daf genes and their human homologs are provided.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000033068	A1	20000608	WO 1999-US28529	19991202

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ,

BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2001029617 A1 20011011 US 1998-205658 19981203
EP 1163515 A1 20011219 EP 1999-960641 19991202
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

L4 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 2002:2553 CAPLUS
DN 136:381187
TI Regulation of C. elegans **DAF-16** and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway
SO Current Biology (2001), 11(24), 1950-1957
CODEN: CUBLE2; ISSN: 0960-9822
AU Lee, Raymond Y. N.; Hench, Jurgen; **Ruvkun, Gary**
AB C. elegans insulin-like signaling regulates metab., development, and life span. This signaling pathway neg. regulates the activity of the forkhead transcription factor **DAF-16**. **Daf-16** encodes multiple isoforms that are expressed in distinct tissue types and are probable orthologs of human FKHRL1, FKHR, and AFX. The authors show that human FKHRL1 can partially replace **DAF-16**, proving the orthol. In mammalian cells, insulin and insulin-like growth factor signaling activate AKT/PKB kinase to neg. regulate the nuclear localization of **DAF-16** homologs. The authors show that the absence of AKT consensus sites on **DAF-16** is sufficient to cause dauer arrest in daf-2(+) animals, proving that **daf-16** is the major output of insulin signaling in C. elegans. FKHR, FKRHL1, and AFX may similarly be the major outputs of mammalian insulin signaling. Daf-2 insulin signaling, via AKT kinases, neg. regulates **DAF-16** by controlling its nuclear localization. Surprisingly, the authors find that daf-7 TGF-.beta. signaling also regulates **DAF-16** nuclear localization specifically at the time when the animal makes the commitment between diapause and reproductive development. **Daf-16** function is supported by the combined action of two distinct promoter/enhancer elements, whereas the coding sequences of two major **DAF-16** isoforms are interchangeable. Together, these observations suggest that the combined effects of transcriptional and posttranslational regulation of **daf-16** transduce insulin-like signals in C. elegans and perhaps more generally.

L4 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 2001:318557 CAPLUS
DN 135:72830
TI Phosphatidylinositol 3-kinase signaling inhibits **DAF-16** DNA binding and function via 14-3-3-dependent and 14-3-3-independent pathways
SO Journal of Biological Chemistry (2001), 276(16), 13402-13410
CODEN: JBCHA3; ISSN: 0021-9258
AU Cahill, Catherine M.; Tzivion, Guri; Nasrin, Nargis; Ogg, Scott; Dore, Justin; **Ruvkun, Gary**; Alexander-Bridges, Maria
AB In Caenorhabditis elegans, an insulin-like signaling pathway to phosphatidylinositol 3-kinase (PI 3-kinase) and AKT neg. regulates the activity of **DAF-16**, a Forkhead transcription factor. We show that in mammalian cells, C. elegans **DAF-16** is a direct target of AKT and that AKT phosphorylation generates 14-3-3 binding sites and regulates the nuclear/cytoplasmic distribution of **DAF-16** as previously shown for its mammalian homologs FKHR and FKHRL1. In vitro, interaction of AKT-phosphorylated **DAF -16** with 14-3-3 prevents **DAF-16** binding to its target site in the insulin-like growth factor binding protein-1 gene, the insulin response element. In HepG2 cells, insulin signaling to PI 3-kinase/AKT inhibits the ability of a GAL4 DNA binding domain/**DAF -16** fusion protein to activate transcription via the insulin-like growth factor binding protein-1-insulin response element, but not the GAL4 DNA binding site, which suggests that insulin inhibits the interaction of **DAF-16** with its cognate DNA site. Elimination of the **DAF-16**/14-3-3 assocn. by mutation

of the AKT/14-3-3 sites in **DAF-16**, prevents 14-3-3 inhibition of **DAF-16** DNA binding and insulin inhibition of **DAF-16** function. Similarly, inhibition of the **DAF-16**/14-3-3 assocn. by exposure of cells to the PI 3-kinase inhibitor LY294002, enhances **DAF-16** DNA binding and transcription activity. Surprisingly constitutively nuclear **DAF-16** mutants that lack AKT/14-3-3 binding sites also show enhanced DNA binding and transcription activity in response to LY294002, pointing to a 14-3-3-independent mode of regulation. Thus, our results demonstrate at least two mechanisms, one 14-3-3-dependent and the other 14-3-3-independent, whereby PI 3-kinase signaling regulates **DAF-16** DNA binding and transcription function.

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FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 11:18:50 ON 16 APR 2003

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L1 76 S E4
L2 69 DUP REM L1 (7 DUPLICATES REMOVED)
L3 11 S L2 AND DAF-16
L4 11 SORT L3 PY

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L4 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS
AN 1995:13340 CAPLUS
DN 122:48240
TI Daf-2, **daf-16** and daf-23: Genetically interacting genes controlling dauer formation in Caenorhabditis elegans
SO Genetics (1994), 137(1), 107-20
CODEN: GENTAE; ISSN: 0016-6731
AU Gottlieb, Shoshanna; **Ruvkun, Gary**
AB Under conditions of high population d. and low food, Caenorhabditis elegans forms an alternative third larval stage, called the dauer stage, which is resistant to desiccation and harsh environments. Genetic anal. of some dauer constitutive (Daf-c) and dauer defective (Daf-d) mutants has revealed a complex pathway that is likely to function in particular neurons and/or responding tissues. Here the authors analyze the genetic interactions between 3 genes which comprise a branch of the dauer formation pathway that acts in parallel to or downstream of the other branches of the pathway, the Daf-c genes daf-2 and daf-23 and the Daf-d gene **daf-16**. Unlike mutations in other Daf-c genes, mutations in both daf-2 and daf-23 cause non-conditional arrest at the dauer stage. The authors epistasis anal. suggests that daf-2 and daf-23 are functioning at a similar point in the dauer pathway. First, mutations in daf-2 and daf-23 are epistatic to mutations in the same set of Daf-d genes. Second, daf-2 and daf-23 mutants are suppressed by mutations in **daf-16**. Mutations in **daf-16** do not suppress any of the other Daf-c mutants as efficiently as they suppress daf-2 and daf-23 mutants. Third, double mutants between either daf-2 or daf-23 and several other daf-d mutants exhibit an unusual interaction. Based on these results, the authors present a model for the function of daf-2, daf-23 and **daf-16** in dauer formation.

L Number	Hits	Search Text	DB	Time stamp
1	10	Ruvkun NEAR Gary	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:06
7	12	daf-16	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/16 11:06
13	11	(US-6225120-$ or US-6472515-$ or US-6319708-$ or US-6135942-$).did. or (US-20010016332-$ or US-20010029617-$ or US-20020037585-$ or US-20030036079-$).did. or (WO-9805761-$ or WO-9851351-$).did. or (WO-200118549-$).did.	USPAT; US-PGPUB; EPO; DERWENT	2003/04/16 11:14

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(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: RUVKUN, Gary; 120 Herrick Road, Newton, MA 02159 (US). MORRIS, Jason; Box 574, Mather House #427, Harvard University, Cambridge, MA 02138 (US). TISSENBAUM, Heidi; 43 Chandler Street, Belmont, MA 02178 (US).

(74) Agent: ELBING, Karen; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).

(81) Designated States: CA, CN, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: AGE-1 POLYPEPTIDES AND RELATED MOLECULES AND METHODS

(57) Abstract

Disclosed are substantially pure AGE-1 polypeptides and purified DNAs, vectors, and cells encoding those polypeptides. Also disclosed are methods for determining longevity and isolating antagonists using the AGE-1 sequence.

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(71) Applicant *(for all designated States except US)*: **NEUROGENETICS, INC.** [US/US]; 4310 La Jolla Village Drive #400, San Diego, CA 92122 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HENDERSON, Samuel, T.** [US/US]; 2110 West 10th Avenue #203, Broomfield, CO 80020 (US). **JOHNSON, Thomas, E.** [US/US]; 1121 West Enclave Circle, Louisville, CO 80027 (US).

(74) Agents: **SWANSON, Barry, J.** et al.; Swanson & Bratschun, L.L.C., Suite 330, 1745 Shea Center Drive, Highlands Ranch, CO 80129 (US).

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Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/18549 A1

(54) Title: THERAPIES AND REAGENTS FOR INCREASING STRESS RESISTANCE AND LIFE SPAN

(57) Abstract: The invention comprises methods for identifying agents that can activate *C. elegans* DAF-16 and human homologs thereof, or their corresponding genes, whereby cytoprotective effects in cells may be induced. Such cytoprotective effects can result in enhanced environmental stress resistance, increased life span and improved late life vigor without significant inhibition of insulin-signaling pathways. The invention also comprises the therapeutic agents identified and methods of treatment using the agents.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/24487

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 51351 A (GEN HOSPITAL CORP) 19 November 1998 (1998-11-19) cited in the application	1-51
Y	the whole document	1,5,6, 10,16, 20,28, 32,47,51

Y	RENA G ET AL: "Phosphorylation of the transcription factor forkhead family member FKHR b protein kinase B." JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 JUN 11) 274 (24) 17179-83. , XP000981982 the whole document	1,5,6, 10,16, 20,28, 32,47,51

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 February 2001	22/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/24487

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	KOPS G J ET AL: "Direct control of the Forkhead transcription factor AFX by protein kinase B." NATURE, (1999 APR 15) 398 (6728) 630-4. , XP000955388 the whole document	1,5,6, 10,16, 20,28, 32,47,51

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/24487

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9851351 A	19-11-1998	AU 7494198 A	08-12-1998
		EP 1019092 A	19-07-2000
		PL 336858 A	17-07-2000
		HU 0002199 A	28-09-2000